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Viral PEG purification

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for viral particle purification via PEG percipitation

Materials

PBS, 0.45 µm syringe filter, 10% w/v PEG-8000, 0.5 M NaCl, 10 mM CaCl₂, 50 mM MgCl₂, DNase I, RNase A



- 1 Add 30 ml of ice cold PBS to 3 g of faecal sample and mix by vortexing at high speed for 5 min, then keep on ice
- 2 Centrifuge sample at 5000 rpm for 10 min at 4°C and transfer the supernatant to a clean 50 ml centrifuge tube
- 3 Filter the supernatant through a 0.45 µm syringe filter
- 4 Add 10 % 10% w/v PEG-8000 in saline (0.5 M NaCl) to filtrate, mix by inverting and incubate at 4°C overnight
- 5 Centrifuge sample at 5000 rpm for 20 min at 4°C and discard the supernatant.
- 6 Re-suspend pellet in 200 µl PBS and transfer it to a 1.5ml Eppendorf tube
- 7 Add an equal volume of chloroform to the sample and mix gently before centrifuging it at 2500 rpm for 5 min at room temperature
- 8 Transfer the aqueous phase into a new and sterile 1.5 ml tube
- 9 Add 40 µl of 10 mM CaCl₂ and 50 mM MgCl₂ to the sample, as well as 10 U of DNase and 10 U of RNase A
- 10 Incubate samples at 37°C for 2 h and then place them in a heatblock at 70°C for 10 min
- 11 Store at 4°C